What is claimed is:

1. A method for determining the presence or absence of a predetermined exogenous nucleic acid target sequence in a nucleic acid sample that comprises the steps of:

(A) providing a treated sample that may contain said predetermined exogenous nucleic acid target sequence hybridized with a nucleic acid probe that includes an identifier nucleotide in the 3'-terminal region;

(B) admixing the treated sample with a depolymerizing amount of an enzyme whose activity is to release one or more nucleotides from the 3'-terminus of a hybridized nucleic acid probe to form a treated reaction mixture;

(C) maintaining the treated reaction mixture for a time period sufficient to permit the enzyme to depolymerize hybridized nucleic acid and release identifier nucleotides therefrom; and

(D) analyzing for the presence of released identifier nucleotides to obtain an analytical output, the analytical output indicating the presence or absence of said exogenous nucleic acid target sequence.

2. The method according to claim 1 wherein said identifier nucleotide is a nucleoside triphosphate.

3. The method according to claim 1 wherein said analytical output is obtained by luminescence spectroscopy.

4. The method according to claim 1 wherein said analytical output is obtained by fluorescence spectroscopy.

5. The method according to claim 1 wherein said analytical output is obtained by mass spectrometry.

6. The method according to claim 1 wherein said analytical output is obtained by absorbance spectroscopy.

7. The method according to claim 1 including the further steps of forming said treated sample by

(A) admixing a sample to be assayed with one or more nucleic acid probes to form a hybridization composition, wherein the 3'-terminal region of said nucleic acid probes (i) hybridize with partial or total complementarity to said exogenous nucleic acid target sequence when that sequence is present in the sample and (ii) include an identifier nucleotide;

(B) maintaining said hybridization composition for a time period sufficient to form a treated sample that may contain said one predetermined exogenous nucleic acid target sequence hybridized with a nucleic acid probe.

8. The method according to claim 1 wherein said nucleic acid sample is obtained from a biological sample.

9. The method according to claim 8 wherein said predetermined exogenous nucleic acid target sequence is a microbial or viral nucleic acid.

10. The method according to claim 9 wherein said predetermined exogenous nucleic acid target sequence is a viral nucleic acid and the magnitude of the analytical output from a predetermined amount of said biological fluid provides a measure of the viral load in the biological sample.

11. The method according to claim 1 wherein said nucleic acid sample is obtained from a food source.

12. The method according to claim 11 wherein said food source is a plant.

13. The method according to claim 12 wherein said predetermined exogenous nucleic acid target sequence is a sequence non-native to the genome of said plant.

14. The method according to claim 13 wherein said sequence non-native to the genome of said plant is a transcription control sequence.

15. The method according to claim 14 wherein said transcription control sequence is that of the 35S promoter or the NOS terminator.

16. The method according to claim 7 including the further steps of preparing a nucleic acid sample to be assayed by amplifying an exogenous nucleic acid sequence from a crude nucleic acid sample.

17. A method for determining the presence or absence of at least one predetermined exogenous nucleic acid target sequence in a nucleic acid sample that comprises the steps of:

(A) admixing a sample to be assayed with one or more nucleic acid probes to form a hybridization composition, wherein the 3'-terminal region of said nucleic acid probes (i) hybridizes with partial or total complementarity to at least one said predetermined exogenous nucleic acid target sequence when that sequence is present in the sample and (ii) includes an identifier nucleotide;

(B) maintaining said hybridization composition for a time period sufficient to form a treated sample that may contain said predetermined exogenous nucleic acid target sequence hybridized with a nucleic acid probe;

(C) admixing the treated sample with a depolymerizing amount of an enzyme whose activity is to release one or more nucleotides from the 3'-terminus of a hybridized nucleic acid probe to form a treated reaction mixture;

(D) maintaining the treated reaction mixture for a time period sufficient to permit the enzyme to depolymerize hybridized nucleic acid and release identifier nucleotides therefrom; and

(E) analyzing for the presence of released identifier nucleotides to obtain an analytical output, the analytical output indicating the presence or absence of at least one said exogenous nucleic acid target sequence.

18. The method according to claim 17 wherein said identifier nucleotide is a nucleoside triphosphate.

19. The method according to claim 17 wherein said analytical output is obtained by luminescence spectroscopy.

20. The method according to claim 17 wherein said analytical output is obtained by fluorescence spectroscopy.

21. The method according to claim 17 wherein said analytical output is obtained by mass spectrometry.

22. The method according to claim 17 wherein said analytical output is obtained by absorbance spectroscopy.

23. The method according to claim 17 wherein said enzyme whose activity is to release nucleotides is a template-dependent polymerase that, in the presence of pyrophosphate ions, depolymerizes hybridized nucleic acids whose bases in the 3'-terminal region are matched with total complementarity.

24. The method according to claim 17 wherein said enzyme whose activity is to release nucleotides exhibits a 3'→ 5' exonuclease activity, depolymerizing hybridized nucleic acids having one or more mismatched bases in the 3'-terminal region of the hybridized probe.

25. A method for determining the presence or absence of an exogenous nucleic acid target sequence containing an interrogation position in a nucleic acid sample that comprises the steps of:

(A) providing a treated sample that contains a nucleic acid sample that may include said exogenous nucleic acid target sequence hybridized with a nucleic acid probe that is comprised of three sections, (i) a first section that contains the probe 3'-terminal about 10 to about 30 nucleotides that are complementary to the exogenous nucleic acid target sequence at positions beginning about 1 to about 30 nucleic acids downstream of said interrogation position of the target sequence, (ii) a 5'-terminal region of about 10 to about 200 nucleic acids in length and having the identical sequence of said

exogenous nucleic acid target sequence, and (iii) an optional third section that contains zero to about 50 nucleic acids that are not complementary to said nucleic acid sample, and ;

(B) extending said nucleic acid probe in a 3' direction to form a second probe hybridized to the nucleic acid sample as a second hybrid;

(D) denaturing said second hybrid to separate said second probe from said exogenous nucleic acid target sequence;

(E) renaturing said aqueous composition to form hairpin structures from said second probe;

(F) admixing the hairpin structure-containing composition with a depolymerizing amount of an enzyme whose activity is to release one or more nucleotides from the 3'-terminus of a nucleic acid hybrid to form a treated reaction mixture;

(G) maintaining the treated reaction mixture for a time period sufficient to permit the enzyme to depolymerize hybridized nucleic acid and release one or more nucleotides from the 3'-terminus therefrom; and

(H) analyzing for the presence of released identifier nucleotide to obtain an analytical output, the analytical output indicating the presence or absence of said exogenous nucleic acid target sequence.

26. A method for determining the presence or absence of an exogenous nucleic acid target sequence, or a specific base within the said target sequence,

in a nucleic acid sample, that comprises the steps of:

(A) providing a treated sample that contains a nucleic acid sample that may include an exogenous nucleic acid target sequence hybridized with a first nucleic acid probe as a first hybrid, said first probe being comprised of at least two sections, a first section containing the probe 3'-terminal about 10 to about 30 nucleotides that are complementary to the target nucleic acid sequence at a position beginning about 5 to about 30 nucleotides downstream of the target interrogation position, a second section of the first probe containing about 5 to about 30 nucleotides that are a repeat of the target sequence from the interrogation position to about 10 to about 30 nucleotides downstream of the interrogation position that does not hybridize to said first section of the probe, and an optional third section of the probe located between the first and second sections of the probe that is zero to about 50 nucleotides in length and comprises a sequence that does not hybridize to either the first or second section of the probe;

(B) extending the first hybrid in the treated sample at the 3'-end of the first probe, thereby extending the first probe past the interrogation position and forming an extended first hybrid that includes an interrogation position;

(C) denaturing an aqueous composition of the extended first hybrid to separate the two nucleic acid strands and form an aqueous composition

containing a separated target nucleic acid and a separated extended first probe;

(D) annealing to the extended first probe a second probe that is about 10 to about 30 nucleotides in length and is complementary to the extended first probe at a position beginning about 5 to about 2000 nucleotides downstream of the interrogation position in the extended first probe, thereby forming a second hybrid;

(E) extending the second hybrid at the 3'-end of the second probe until that extension reaches the 5'-end of the extended first probe, thereby forming a second extended hybrid containing a second extended probe whose 3'-region includes an identifier nucleotide;

(F) denaturing an aqueous composition of the extended second hybrid to separate the two nucleic acid strands and form an aqueous composition containing separated extended first and second probes;

(G) cooling the aqueous composition to form a hairpin structure from the separated extended second probe to form a hairpin structure-containing composition;

(H) admixing the hairpin structure-containing composition with a depolymerizing amount of an enzyme whose activity is to release one or more nucleotides from the 3'-terminus of a nucleic acid hybrid to form a treated reaction mixture;

(I) maintaining the reaction mixture for a time period sufficient to release 3'-terminal region identifier nucleotides; and

(J) analyzing for the presence of released identifier nucleotide to obtain an analytical output, the analytical output indicating the presence or absence of said predetermined exogenous nucleic acid target sequence or a specific base within the target sequence.

27. The method according to claim 26 wherein said analytical output is obtained by luminescence spectroscopy.

28. The method according to claim 26 wherein said analytical output is obtained by fluorescence spectroscopy.

29. The method according to claim 26 wherein said analytical output is obtained by mass spectrometry.

30. The method according to claim 26 wherein said analytical output is obtained by absorbance spectroscopy.

31. A method for determining the presence or absence of a specific base in an exogenous nucleic acid target sequence in a sample to be assayed that comprises the steps of:

(A) admixing a sample to be assayed with one or more nucleic acid probes to form a hybridization composition, wherein the 3'-terminal region of at least one of said nucleic acid probes (i) is substantially complementary to said nucleic acid target sequence and comprises at least one predetermined nucleotide at an interrogation position, and (ii) includes an identifier nucleotide, and wherein said nucleic acid target sequence comprises at least one specific base whose presence or absence is to be determined

(B) maintaining said hybridization composition for a time period sufficient to form a treated sample, wherein said interrogation position of the probe is a nucleotide that is aligned with said specific base to be identified in said target sequence, when present, so that base pairing can occur;

(C) admixing the treated sample with an enzyme whose activity is to release one or more nucleotides from the 3'-terminus of a hybridized nucleic acid probe to depolymerize the hybrid and form a treated reaction mixture;

(D) maintaining the treated reaction mixture for a time period sufficient to release an identifier nucleotide therefrom; and

(E) analyzing for the presence or absence of released identifier nucleotide to obtain an analytical output that indicates the presence or absence of said specific base to be identified.

32. The method according to claim 31 wherein the identifier nucleotide is at the interrogation position.

33. The method according to claim 31 wherein said analytical output is obtained by fluorescence spectroscopy.

34. The method according to claim 31 wherein said analytical output is obtained by mass spectrometry.

35. The method according to claim 31, wherein said nucleic acid target sequence is selected from the group consisting of deoxyribonucleic acid and ribonucleic acid.

36. The method according to claim 35, further comprising a first probe, a second probe, a third probe and a fourth probe.

37. A one-pot method for determining the presence or absence of at least one predetermined exogenous nucleic acid target sequence in a nucleic acid sample that comprises the steps of:

(A) admixing a treated sample that may contain said predetermined nucleic acid target sequence hybridized to a nucleic acid probe whose 3'-terminal region is completely complementary to said predetermined nucleic acid target sequence and includes an identifier nucleotide with (i) a

depolymerizing amount of an enzyme whose activity in the presence of pyrophosphate is to release identifier nucleotide as a nucleoside triphosphate from the hybridized nucleic acid probe, (ii) adenosine 5' diphosphate, (iii) pyrophosphate and (iv) NDPK to form a treated reaction mixture;

(B) maintaining the treated reaction mixture at a temperature of about 25 to about 80 degrees C for a time period sufficient to permit the enzyme to depolymerize hybridized nucleic acid probe, release an identifier nucleotide in the 3'-terminal region as a nucleoside triphosphate and to convert said nucleoside triphosphate and said adenosine 5' diphosphate to adenosine 5' triphosphate; and

(C) analyzing for the presence of adenosine 5' triphosphate to obtain an analytical output, the analytical output indicating the presence or absence of at least one said nucleic acid target sequence.

38. The method according to claim 37 wherein said analytical output is obtained by luminescence spectroscopy.

39. The method according to claim 37 including the further steps of forming said treated sample by

(A) admixing a sample to be assayed with one or more nucleic acid probes to form a hybridization composition, wherein the 3'-terminal region of said nucleic acid probe (i) hybridizes with partial or total complementarity to a nucleic acid target

sequence when that sequence is present in the sample and (ii) includes an identifier nucleotide;

(B) maintaining said hybridization composition for a time period sufficient to form a treated sample that may contain said one predetermined nucleic acid target sequence hybridized with a nucleic acid probe.

40. The method according to claim 37 wherein said depolymerizing enzyme maintains activity at 60-90°C.

41. The method according to claim 40 wherein said depolymerizing enzyme is a thermostable polymerase.

42. The method according to claim 37 wherein said NDPK is that encoded by *Pyrococcus furiosis*.

43. A kit for determining the presence or absence of a predetermined exogenous nucleic acid target sequence in a nucleic acid sample comprising:

(A) a purified and isolated enzyme whose activity is to release one or more nucleotides from the 3′ terminus of a hybridized nucleic acid probe; and

(B) a nucleic acid probe, said nucleic acid probe being complementary to a predetermined exogenous nucleic acid target sequence.

44. The kit according to claim 43 wherein the predetermined exogenous nucleic acid probe sequence is species-specific.

45. The kit according to claim 44 wherein the nucleic acid probe comprises one of the following nucleic acid sequences or their complementary sequences:

5' CCAGACGCCTCA 3' SEQ ID NO:86;
5' ACCTTCACGCCA 3' SEQ ID NO:87;
5' TGCCGAGACGT 3' SEQ ID NO:88;
5' GCAGACACATCC 3' SEQ ID NO:89;
5' GGAATCTCCACG 3' SEQ ID NO:90;
5' ACATACACGCAA 3' SEQ ID NO:91; and
5' ATATGCACGCAA 3' SEQ ID NO:92.

46. The kit according to claim 43 wherein the predetermined nucleic acid target sequence is associated with a pathogen.

47. The kit according to claim 46 wherein the nucleic acid probe comprises one of the following nucleic acid sequences or their complementary sequences:

5'CGTTGTGCGGGTTCACGTCGATGAGCACGT
TCATGGGTGTAATATCAAAGTGGCATACACGAGCT 3' SEQ ID NO:82

5'CACTTTGATATTACACCCATG 3' SEQ ID NO:35

5' TCACACAGGAAACAGCTATGACCATG 3' SEQ ID NO:41

5′ CTGCTAGCCGAGTAGTGTTGGGTCGCGAAAGGCCTTGTGG 3′
SEQ ID NO:43

5' CCATTTAGTACTGTCT 3' SEQ ID NO:52

5' CTAGTTTTCTCCATTT 3' SEQ ID NO:54

5' TTCTCTGAAATCTACT 3' SEQ ID NO:56

5' AAAAAAGACAGTACTAAATGGAGAAAACTAGTA
GATTTCAGAGAACTTAA 3' SEQ ID NO:58

5′ CACTTTGATATTACACCCGTG 3′ SEQ ID NO:36

5'CGTGTATGCCACTTTGATATTACACCCGTGAACGTGCTCATCGACGTGAAC
CCGCACAACGAGCT 3' SEQ ID NO:83

5'CGTTGTGCGGGTTCACGTCGATGAGCACGTTCACGGGTGTAATATCAAAGT
GGCATACACGAGCT3' SEQ ID NO:84

5′CGCTTCTACCACGAATGCTCGCAGACCATGCTGCACGAAT
ACGTCAGAAAGAACGTGGAGCGTCTGTTGGAGCT 3′ SEQ ID NO:1

5′CCAACAGACGCTCCACGTTCTTTCTGACGTATTCGTGCAGC
ATGGTCTGCGAGCATTCGTGGTAGAAGCGAGCT 3′ SEQ ID NO:2

5′CGCTTCTACCACGAATGCTCGCAGATCATGCTGCACGAAT
ACGTCAGAAAGAACGTGGAGCGTCTGTTGGAGCT 3′ SEQ ID NO:3

5′CCAACAGACGCTCCACGTTCTTTCTGACGTATTCGTGC

AGCATGATCTGCGAGCATTCGTGGTAGAAGCGAGCT 3' SEQ ID NO:4

5' AAAAAAAACAGTACTAAATGGAGAAAACTAGTAGA
TTTCAGAGAACTTAA 3' SEQ ID NO:59

5'AAAAAAGACAGTACTAGATGGAGAAAACTAGTAGATTTCAG
AGAACTTAA 3' SEQ ID NO:60

5'AAAAAAGACAGTACTAAATGGAGAAAACTAA
TAGATTTCAGAGAACTTAA 3' SEQ ID NO:61

5' TTCTCTGAAATCTATT 3' SEQ ID NO:57

5' CTAGTTTTCTCCATCT 3' SEQ ID NO:55

5' CCATTTAGTACTGTTT 3' SEQ ID NO:53

5' GAAGTAAAACAAACTACACAAGCAACTACACCTGCGCCTAAAG
TAGCAGAAACGAAAGAAACTCCAGTAG 3' SEQ ID NO:9

5' CTACTGGAGTTTCTTTCGTTTCTGCTACTTTAGGCGCAGGT
GTAGTTGCTTGTGTAGTTTGTTTTACTTC 3' SEQ ID NO:10

5' GCAACTACACCTGCGCCTAAAGTAGCAGAA 3'SEQ ID NO:11

5' TTCTGCTACTTTAGGCGCAGGTGTAGTTCG 3'SEQ ID NO:12

5' CATCGACGGCAACCTCGGAGACTTACGAGATATTTTGAAAAAA
GGCGCTACTTTTAATCGAGAAACACCA 3' SEQ ID NO:13

5' TGGTGTTTCTCGATTAAAAGTAGCGCCTTTTTTCAAAATATCT

CGTAAGTCTCCGAGGTTGCCGTCGATG 3' SEQ ID NO:14

5' CTCGGAGACTTACGAGATATTTTGAAAAAA 3' SEQ ID NO:15

5' TTTTTTCAAAATATCTCGTAAGTCTCCGAG 3' SEQ ID NO:16

5' TGTGTAATGAAAGAAATCACCGTCACTGAA 3'SEQ ID NO:19

5' TTCAGTGACGGTGATTTCTTTCATTACACA 3'SEQ ID NO:20

5'CTTGAAGCATAGTTCTTGTTTTTAAACTTTGTCCATCTT GAGCCGCTTGAGTTGCCTTAGTTTTAATAGT 3' SEQ ID NO:31

5'ACTATTAAAACTAAGGCAACTCAAGCGGCTCAAGATGGACAAAGTTTAAAA ACAAGAACTATGCTTCAAG 3' SEQ ID NO:33

5'AGTTCTTGTTTTTAAACTTTGTCCATCTTG 3' SEQ ID NO:32

5'CAAGATGGACAAAGTTTAAAAACAAGAACT 3' SEQ ID NO:34

48. The kit according to claim 43 wherein said nucleic acid probes comprise a fluorescent label.

49. The kit according to claim 43 wherein said nucleic acid probes comprise a non-natural nucleotide analog.

50. The kit according to claim 43 further comprising pyrophosphate.

51. The kit according to claim 43 further comprising a nucleotide diphosphate kinase.

52. The composition according to 51, wherein said nucleoside diphosphate kinase is that encoded by *Pyrococcus furiosis*.

53. A composition for determining the presence or absence of a plurality of predetermined nucleic acid target sequences in a nucleic acid sample comprising an aqueous solution that contains:

(A) a purified and isolated enzyme whose activity is to release one or more nucleotides from the 3′ terminus of a hybridized nucleic acid probe; and

(B) a plurality of nucleic acid probes, each of said nucleic acid probes being complementary to a predetermined nucleic acid target sequence.

54. A composition of matter for determining the presence or absence of a plurality of predetermined nucleic acid target sequences in a nucleic acid sample comprising an aqueous solution that contains:

(A) a purified and isolated enzyme whose activity in the presence of pyrophosphate is to release identifier nucleotide as a nucleoside triphosphate from hybridized nucleic acid probe;

(B) adenosine 5′ diphosphate;

(C) pyrophosphate;

(D) a purified and isolated nucleoside diphosphate kinase; and

(E) a plurality of nucleic acid probes, each of said nucleic acid probe being complementary to its respective predetermined nucleic acid target sequence.

55. The composition of matter according to claim 181, wherein said purified and isolated enzyme whose activity in the presence of pyrophosphate is to release identifier nucleotides is a thermostable polymerase.

56. The composition of matter according to claim 53, wherein said purified and isolated nucleoside diphosphate kinase is that encoded by *Pyrococcus furiosis*.

57. A method for determining the presence or absence of a first exogenous nucleic acid target in a nucleic acid sample containing that target or a substantially identical second exogenous target that comprises the steps of:

(A) admixing said sample to be assayed with one or more nucleic acid probes to form a hybridization composition, wherein said first and second exogenous nucleic acid targets comprise a region of sequence identity except for at least a single nucleotide at a predetermined position that differs between the targets, and wherein said nucleic acid probe (i) is substantially complementary to said nucleic acid

target region of sequence identity and comprises at least one nucleotide at an interrogation position, said interrogation position of the probe being aligned with said predetermined position of a target when a target and probe are hybridized and (ii) includes an identifier nucleotide in the 3'-terminal region;

(B) maintaining said hybridization composition for a time period sufficient to form a treated sample wherein the nucleotide at said interrogation position of said probe is aligned with the nucleotide at said predetermined position of said target in said region of identity;

(C) admixing the treated sample with a depolymerizing amount an enzyme whose activity is to release one or more nucleotides from the 3'-terminus of a hybridized nucleic acid probe to form a treated reaction mixture;

(D) maintaining the treated reaction mixture for a time period sufficient to release identifier nucleotide and depolymerize said hybridized nucleic acid probe; and

(E) analyzing for the presence of released identifier nucleotide to obtain an analytical output, said analytical output indicating the presence or absence of said nucleotide at said predetermined region and thereby the presence or absence of a first or second exogenous nucleic acid target.